EFFECT OF INHIBITORS ON ACTIVE TRANSPORT BY TURTLE INTESTINAL SEGMENTS [1]

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Intestinal absorption of sugars against a concentration gradient has been shown to occur in cold-blooded as well as warm-blooded animals (Csaky and Fernald, 1960; Fox, 1961a, 1961b; Lawrence, 1963; Musacchia *et al.*, 1964). The studies reported here used a number of inhibitors to reveal the energy source for glucose absorption by intestinal segments (*in vitro*) of the painted turtle, *Chrysemys picta*.

Materials and Methods

Painted turtles (5–6" shell length) of both sexes were obtained commercially. They were kept at room temperature during all seasons, and were fed pieces of horsemeat once each week. The animals were sacrificed by severing the spinal cord with a bone shears. Segments 6 cm. in length were dissected from the upper small intestine and sacs prepared using the method of Crane and Wilson (1958). This method calls for eversion of each segment so that the mucosal epithelium is "outside" and transport of materials is from the outer surface toward the inner.

The incubation medium consisted of Krebs-Ringer-bicarbonate (NaCl = 0.7%) plus 4.5 mg./100 ml. D-glucose (2.5×10^{-4} *M*), with or without inhibitor. One ml. of this solution was placed inside the sac (serosal side) and the preparation was immersed in 10 ml. of the same solution (mucosal side). A control segment and one or two experimental segments from the same animal were incubated simultaneously in a Dubnoff shaking water bath in separate Erlenmeyer flasks for 60 minutes at 30° C. This temperature was chosen because previous work with *Chrysemys picta* (Fox, 1961b) had shown the mucosal uptake and serosal accumulation of D-glucose to be at a maximum at 30° C. Air or nitrogen was bubbled through the medium on the mucosal side during incubation. After 60 minutes, 0.5-ml. samples of the mucosal and serosal solutions were analyzed for their glucose concentration, using the method of Nelson (1944) or the glucose oxidase reaction.

The following compounds at different concentrations were added to the incubation medium in separate experiments: potassium salt of monoiodoacetic acid (IAA), 2,4-dinitrophenol (DNP), phlorizin, disodium salt of malonic acid, sodium azide, sodium cyanide and ouabain. In one series of experiments nitrogen rather than air was bubbled through the mucosal fluid.

Each experimental run had its own control segment; mucosal uptake of D-glucose by the segments exposed to an inhibitor was compared to mucosal uptake by the control segment taken from the same animal. Segments were weighed and wet weights recorded at the end of each run. The amounts of glucose taken up

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TABLE I

*Inhibition (%) of active transport of D-glucose by isolated intestinal segments of Chrysemys picta**

Inhibitor	10^{-1} *M*	10^{-2} *M*	10^{-3} *M*	10^{-4} *M*	10^{-6} *M*	Number of segments used
Azide		0	0	0		32
Cyanide	70	60	15	0		51
Dinitrophenol	100	80	15	0		24
Iodoacetate		70	50	10	0	26
Malonate	70	50	0	0		27
Ouabain		90	75	50	90	23
Phlorizin			100	80	100	32
Nitrogen	No significant % of inhibition.					12

* All experiments were aerobic except those using nitrogen.

by the mucosa and added to the serosal side in micromoles per gram tissue (wet wt.) per hour were calculated and these data used to find the percentage of inhibition.

RESULTS

Table I shows the percentage of inhibition observed with different molar concentrations of the compounds tested. One hundred per cent inhibition indicates no net uptake of glucose from the mucosal solution; 0% inhibition indicates that uptake was statistically identical with controls. Phlorizin, iodoacetate and ouabain were potent inhibitors of glucose absorption at concentrations of 10^{-3} *M* or lower, while dinitrophenol, malonate, cyanide and azide were less effective.

When intestinal segments were exposed to 10^{-6} *M* IAA with nitrogen gas instead of air bubbled through the solution, mucosal uptake of glucose was completely inhibited (Table II). This is in contrast to the absence of any inhibitory effect in the presence of IAA (10^{-6} *M*) under aerobic conditions (Table I).

TABLE II

Effect of IAA and nitrogen gas on active transport of D-glucose (initial concentration on both sides = 4.5 mg./100 ml.) by intestinal segments (in vitro) of Chrysemys picta

	μMoles D-glucose/gm. tissue (wet wt.)/hr.		Number of segments used
	Mucosal solution	Serosal solution	
Air bubbled through mucosal solution	−2.2±0.51*	+0.08±0.01	12
Nitrogen gas bubbled through mucosal solution	−2.5±0.38*	+0.20±0.12	8
IAA (1×10^{-6} *M*) + Air	−2.0±0.61*	+0.10±0.01	8
IAA (1×10^{-6} *M*) + Nitrogen	+1.3±0.29*	+0.75±0.15	8

* Mean and the standard error of the mean.

Discussion

Inhibitors are known to act at different sites to block the active transport of sugars by intestinal tissue (Darlington and Quastel, 1953; Crane, 1960).

Phlorizin

Phlorizin inhibits glucose transport systems in both kidney tubules and intestinal tissues (Nakazawa, 1922; Jolliffe *et al.*, 1932; Jervis *et al.*, 1956). At a concentration of 10^{-6} *M*, phlorizin prevents entry of glucose into the mucosal cells, while higher concentrations interfere with the cellular oxidation of glucose (Parsons *et al.*, 1958; Ponz and Balasch, 1964). According to Alvarado and Crane (1962) phlorizin blocks glucose absorption by competitive inhibition for a "transport site" on a carrier in the brush border.

The studies reported here demonstrate transport inhibition by phlorizin at a concentration of 10^{-6} *M*, as well as the more generalized effect on cell metabolism at concentrations of 10^{-4} *M* and higher. This suggests that turtle mucosal cells contain a carrier or transport site similar to that of higher vertebrates.

Iodoacetate

At low concentrations, iodoacetate is apparently selective in its inhibition of the enzymes of glycolysis, specifically triosephosphate dehydrogenase and phosphofructokinase (Webb, 1963). Working with iodoacetate-poisoned turtles (*Sternothoerus minor*), Belkin (1962) demonstrated a greatly decreased tolerance to anoxia, and concluded that glycolysis is a necessary source of metabolic energy for animals under these conditions. In the present study, when air was replaced by nitrogen during incubation of IAA-treated segments, total inhibition was observed (Table II). These data may be interpreted as further evidence that the turtle can use energy derived from anaerobic glycolysis to drive its transport mechanism.

2,4-Dinitrophenol

Compounds such as 2,4-dinitrophenol are known to depress formation of ATP by dissociating the reactions of phosphorylation from those of electron transfer. This uncoupling phenomenon may be demonstrated at a DNP concentration (10^{-4} *M*) which is ineffective in bringing about any general respiratory inhibition. DNP inhibits the active transport of glucose by rat intestinal segments at 10^{-4} *M* (Darlington and Quastel, 1953) but was found to be ineffective with our turtle preparations. The inhibitory effects at higher DNP concentrations (Table I) may be ascribed to the generalized effect on cell metabolism. We conclude that oxidative phosphorylation is not a necessary source of the energy for absorption of glucose against a concentration gradient by turtle intestinal segments.

Ouabain

A number of investigators (Weatherall, 1960; Csaky *et al.*, 1961; Parkinson, 1964) have shown that the cardiac glycosides exert an inhibitory effect on cellular transport of sodium and potassium ions. At concentrations of 10^{-5} *M* or less, the

effect appears to be at some site on the cell membrane, but at 10^{-3} *M* or higher, these compounds serve to uncouple oxidative phosphorylation, and a specific effect on the sodium pump cannot be demonstrated. Table I shows an inhibitory effect of the glycoside ouabain at a concentration of 10^{-6} *M*. These data support the hypothesis of Crane *et al.* (1961), Crane, (1962) and Csaky (1963) that a sodium transport system is associated with sugar transport, and serve as further evidence of the basic similarity of transport mechanisms in all types of vertebrates.

Azide, cyanide and malonate

Azide and cyanide compounds act to inhibit metallo-enzymes such as cytochrome oxidase. Darlington and Quastel (1953) reported complete inhibition of active transport of glucose by rat intestine by 10^{-2} *M* concentrations of sodium azide and sodium cyanide. Table I shows that turtle intestinal absorption was only 60% inhibited by cyanide at this concentration and was insensitive to azide. These data reinforce the hypothesis that oxidative respiration *via* the cytochrome chain is not essential for intestinal active transport of glucose in *Chrysemys picta*. Neither does the Krebs cycle appear to be essential, since malonate caused only 50% inhibition even at a relatively high concentration (10^{-2} *M*).

Lowered oxygen tension

Several recent publications have documented the fact that turtles are highly tolerant of anoxic conditions such as those normally encountered by these animals in diving and during periods of cold torpor (Belkin, 1963; Klahr and Bricker, 1964; Musacchia *et al.*, 1964; Robin *et al.*, 1964). It appears that turtle cell respiration is able to shift from aerobic to anaerobic pathways in reaction to lowered intracellular oxygen tension or inhibition of the cytochrome system. In such situations the reactions of glycolysis become the prime source of metabolically useful energy.

The concept of turtle metabolism which emerges from these studies is one of flexibility and tolerance to conditions where oxygen is in short supply. We conclude that the turtle may use oxidative pathways but is partially, and at times entirely, dependent upon glycolysis for the energy required for intestinal active transport of sugars.

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Summary

1. Isolated segments of turtle (*Chrysemys picta*) intestine were exposed to metabolic inhibitors and the effects on active transport of D-glucose (4.5 mg./100 ml.) were studied.

2. Phlorizin, iodoacetate and ouabain were potent inhibitors; 2,4-dinitrophenol, malonate, cyanide and azide were less effective.

3. No significant inhibition was observed when nitrogen replaced aeration of the segments, except when segments were simultaneously treated with iodoacetate.

4. The energy for the sugar transport mechanism in this animal appears to come from glycolysis.

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